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REVIEW

Neuropeptides in Neurosecretory and Efferent Neural Systems of Insect Thoracic and Abdominal Ganglia

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INTRODUCTION

Over the last ten years there has been a dramatic increase in the number of identified insect neuropeptides [38, 39, 55, 56, 63, 94]. Many of these peptides have been attributed biological actions in a variety of assays, and it is clear that insect neuropeptides play important roles in different regulatory processes involved in development, reproduction, diapause, metabolism, osmoregulation, muscle activity and behavior [38, 55, 56, 86, 90, 95, 105]. With the development of further bioassays and extensive testing of novel neuropeptides even in heterologous bioassays, it has become increasingly apparent that neuropeptides have more actions that those ascribed to them at the time of isolation [22, 56, 62, 94]. In fact, it may well be that it is a rule rather than an exception that neuropeptides have several functions and thus many names given to neuropeptides may become misleading as novel important functions are revealed.

Classically the isolation of peptides involved ablation of endocrine organs followed by reconstitution of regulatory functions by injections of purified "factors" extracted from these organs. Most insect neuropeptides have, however, been isolated with the aid of *in vitro* assays of actions on peripheral target organs. In either case it is likely that *in vivo* many physiological actions of these peptides are of hormonal nature. Hence, it is not surprising that a large number of insect neuropeptides have been demonstrated by immunocytochemistry in neurosecretory cells and neurohemal release organs [40, 66, 86]. In addition many of the same neuropeptides are present in central neurons and in endocrine cells of the gastro-intestinal tract (reviewed in Refs [18, 66, 94, 110]) indicating that also in insects peptides can act as neuromodulators and local neurohormones [80, 90]. Immunocytochemistry has proved to be a powerful technique for the localization of storage and release sites of both neuropeptides and other neuroactive compounds such as monoamines and amino acid transmitters. By now quite a number of neuropeptides have been mapped in the nervous and neuroendocrine systems of different insects and it is apparent that the complexity in peptidergic signalling is staggering [18, 66, 86, 94]. The number of known insect neuropeptide sequences by far exceed 100 [56, 66, 94]. Only from a single species of insect, *Locusta migratoria,* not less than 32 different neuropeptides have been isolated and sequenced as of mid 1993 and many more are under way [94]. Just a fraction of these have been mapped by immunocytochemistry and almost nothing is known about their receptors and physiological actions in the nervous system and at peripheral targets. For most peptides suspected to be acting as neurohormones it remains to demonstrated that they are actually released into the circulation.

Actions of neuropeptides can be assayed more conveniently at peripheral targets than in the central nervous system. To draw attention to possible peripheral targets for studies of peptide action, the present review focuses on neuropeptides in the neurosecretory and efferent systems of the thoracic and abdominal ganglia of insects, with special emphasis on blowflies. The distribution of neuropeptides in the insect brain [66] and intestine [110] has recently been reviewed.

ORGANIZATION OF INSECT NEUROSECRETORY SYSTEMS

Neurosecretory cells have been classified into a few main groups [86]: (i) neurosecretory cells with cell bodies in central ganglia and axon terminals in peripheral neurohemal organs or release areas, (ii) central neurosecretory cells with peripheral so called neuroeffector junctions (secretory-motorneurons and other efferents with peripheral innervation areas), (iii) central neurosecretory cells with arborizations ("neurosecretory endings") in neuropils of the central nervous system and (iv) peripheral neurosecretory cells with peripheral release sites. Some of this organization is illustrated in Figures 1 and 2.

The first type of neurosecretory cells are found in a few locations of the cephalic ganglia and in the subesophageal, thoracic and abdominal ganglia and send axons to neurohe-

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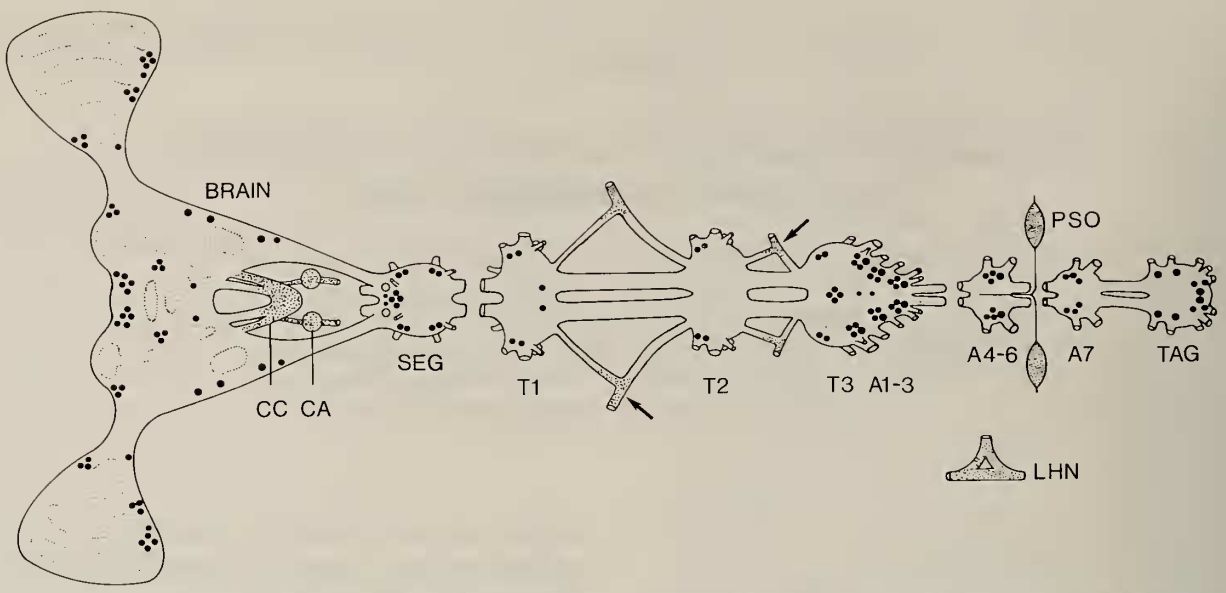

Fig. 1. Distribution of CCAP immunoreactive neurons (black circles) and some neurohemal areas (shaded) in the nervous system of the locust. The neurohemal areas are not drawn for all segments. Arrows point at neurohemal areas in junctions of nerves 1 and 6. Abbreviations: CC=corpora cardiaca, CA=corpora allata, SEG=subesophageal ganglion, T1–T3=thoracic ganglia, A1–A7=abdominal ganglia, TAG=terminal abdominal ganglion, PSO=perisympathetic organ, LHN=lateral heart nerve. From Dircksen *et al.* [19] with permission from Springer Verlag.

mal organs or release areas associated with these ganglia [86]. The cephalic neurosecretory cells supply axons to the neurohemal organs termed corpora cardiaca and corpora allata (Fig. 1) and in some insect species to neurohemal areas in the wall of the anterior aorta (see Fig. 4), in the so called antennal heart and at the surface of certain cranial nerves. The neurosecretory cells of the ventral cord supply segmental neurohemal organs, termed perisympathetic or perivisceral organs, located in the median and/or transverse nerves (Figs. 1–3). Other release sites of ventral cord neurosecretory cells are found in neurohemal areas in the pericardial septum of the abdominal aorta, the lateral cardiac nerve, the dorsal diaphragm and the intestine (Figs. 2, 3).

The "neurosecretory cells" with arborizations in central neuropil and the efferents with targets such as glands and different types of muscle were originally identified with classical neurosecretory staining methods (see Ref. [86]). Some of these cells have later been identified by immunocytochemistry as peptidergic and/or monoaminergic neurons. An example of this kind of cells is the pair of vasopressin immunoreactive neurons of the locust subesophageal ganglion with extensive arborizations restricted to the central nervous system and the core of some peripheral nerve roots [89, 100].

Peripheral neurosecretory cells have been found at several locations: in nerves (link nerves, connecting transverse and segmental nerves) of the thoracic and abdominal ganglia and in nerves associated with the heart and alary muscles and the intestinal tract [28, 86]. Neurosecretory cells have also been reported in the frontal and hypocerebral ganglia.

We shall be concerned here with the neurohemal release sites of the body segments only since the cephalic neurohemal organs have been more frequently dealt with in the literature [33, 40, 56, 86]. In the less evolved insects, such as the locust, the distinct segmental neurohemal organs associated with the dorsal median and transverse nerves are easily distinguished in the larval and adult stages [86] (Figs. 1, 2). In blowflies and other higher diptera, however, these neurohemal organs can only be clearly distinguished before metamorphosis in the larval stages [72] (see Fig. 10). In the adult flies the terminals of the neurosecretory cells are located in the dorsal neural sheath of the fused thoracic and abdominal ganglia [31, 68, 72] (Fig. 4). These "centralized" neurohemal structures represent the most evolved type of neurohemal perisympathetic organs associated with the ventral nerve cord of insects. Intermediate types of organs derived from median or transverse nerve types that anastomose with connectives or segmental nerves are found in hymentopterans, some coleopterans and orthopterans [32, 86].

NEUROPEPTIDES DEMONSTRATED IN THE THORACIC AND ABDOMINAL GANGLIA

Most insect neuropeptides known today have been isolated from whole heads, whole insects, dissected brains and corpora cardiaca-corpora allata complexes or from dissected entire nervous systems [39, 55, 63, 94, 97]. Some neuropeptides have, however, specifically been isolated from dissected thoracic-abdominal ganglia as is the case for some blowfly peptides: thirteen different FMRFamide-related peptides (FaRPs) and two callatostatins, peptides structurally closely related to the cockroach allatostatins [22, 24]. As

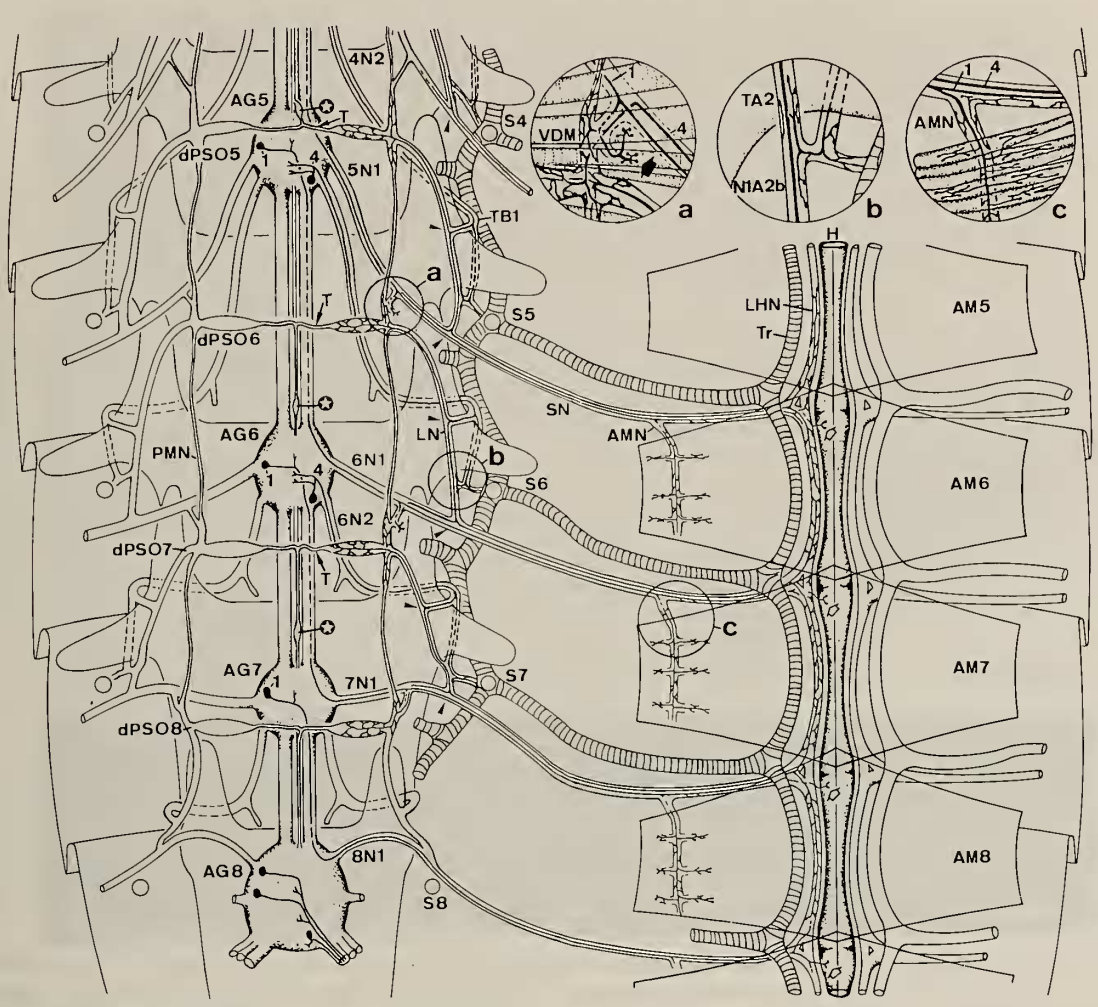

FIG. 2. Semischematic drawing of pathways of CCAP immunoreactive (CCAP-IR) neurons and neurohemal release sites in the locust abdomen. Of interest here are the segmentally repeated neurons 1 and 4 which send axons to segmental nerves 1 and 2 (N1 and N2). From these nerves CCAP-IR supply terminals to neurohemal distal perisympathetic organs (dPSO 5–8), to stigmata of tracheal system (S4–8; *inset circle b*), ventral diaphagm muscles (VDM; *inset circle a*), alary muscles (AM5–8; *inset circle c*) and lateral heart nerve (LHN). No CCAP-IR fibers were seen in median PSOs (asterisks). This drawing is useful as a basis for the organization of primitive and segmental neurohemal structures in insects. Many of these structures are fused in the higher dipteran insects. From Dicksen *et al.* [19] with permission from Springer Verlag.

indicated by immunocytochemistry it appears that most peptides isolated from whole insects, whole heads, whole CNS or dissected brain-corpora cardiaca are present in the ventral cord ganglia. The neuropeptides indicated by immunocytochemistry in the thoracic-abdominal ganglion of blowflies (*Calliphora vomitoria* and *Phormia terraenovae*) are listed in Table 1. In other insect species the presence of some additional native neuropeptides have been indicated in the ventral ganglia by immunocytochemistry. In *Locusta migratoria*: crustacean cardioactive peptide (CCAP) [19], locustamyotropin [94]; male accessory gland myotropin [81], ovary maturating neurohormone [89]. In cockroaches: proctolin [25, 80], leucokinins [70]. In moths: pheromone-biosynthesis-activating neuropeptide (PBAN) in *Helicoverpa zea* [43] and eclosion hormone in *Manduca sexta* larvae [103]. It should be noted that the above mentioned neuropeptides are commonly found not only in neurosecretory cells, but also in different types of interneurons of the thoracic and abdominal ganglia (Table 1). In addition proctolin has been demonstrated in motorneurons [80] and leucokinin in putative sensory neurons (intestinal stretch receptors) [70].

GENERAL ORGANIZATION OF THORACIC-ABDOMINAL NEUROSECRETORY SYSTEMS AND NEUROHEMAL ORGANS AND RELEASE SITES

Before turning to the neurosecretory systems of the fused ventral cord of higher Diptera it may be useful to present the organization of the less evolved neurosecretory system of the locust where the segmental peptidergic neurons and neurohemal structures are clearly discernible. The

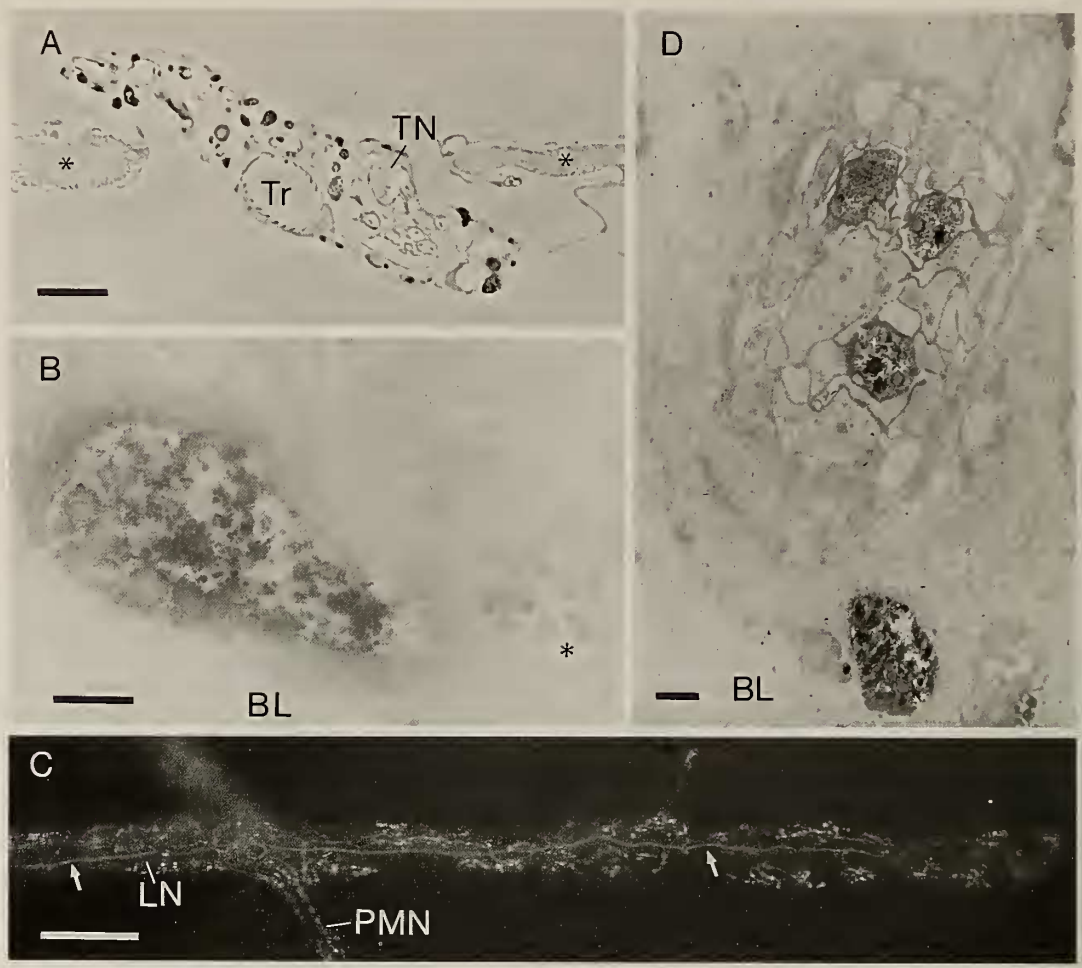

Fig. 3. CCAP immunoreactive structures in sections and whole mount preparations of *Locusta migratoria*. **A.** labeled semithin cross-section through seventh distal perisympathetic organ (dPSO7), showing axon profiles and terminals. Note lack of label in motor axons of the transverse nerve (TN); Ventral diaphragm muscles are labeled by asterisks. Tr= trachea. **B.** Axon terminal in a dPSO7 containing neurosecretory granules (pre-embedding immunocytochemistry; peroxidase labeling). Note unlabeled axon profile (asterisk). BL=basal lamina. **C.** *In situ* whole mount immunofluorescence preparation of a dPSO5 showing and axon originating in the link nerve (LN; arrow) that gives rise to fine terminals at the surface of the dPSO and the paramedian nerve (PMN). **D.** Cross section through the neurohemal lateral heart nerve showing three labeled central axons and axon profiles next to the surface of the nerve. Note the granule contents of almost all profiles. BL=basal lamina. Scales: A=10 μm, B=500 nm, C=100 μm, D=500 nm.

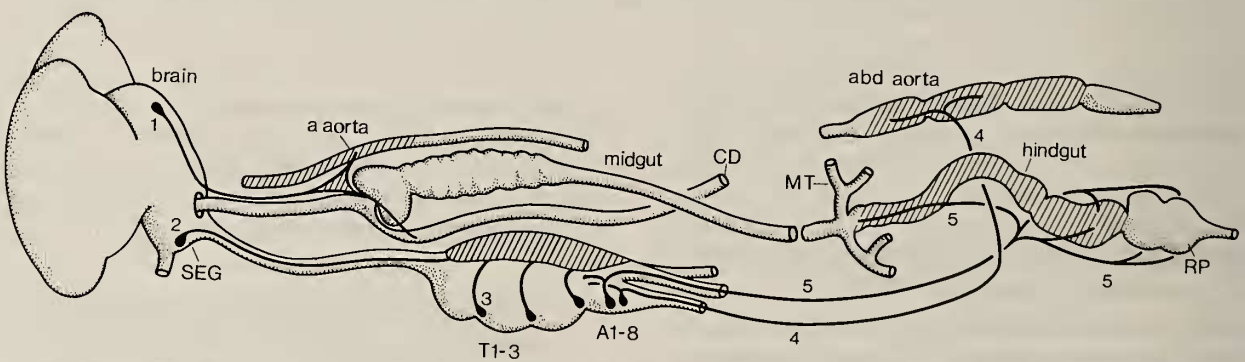

Fig. 4. Schematic dissected view of the nervous system, intestinal tract and aorta of the blowfly. The cross hatched areas are putative release sites of peptidegic neurons. In most cases these areas probably represent neurohemal release sites. Release sites are found in the anterior aorta (a. aorta), corpora cardiaca (triangular cross hatched structure below anterior aorta), dorsal sheath of thoracic-abdominal ganglion (T1–3, A1–8), pericardial septum at posterior aorta (abd aorta) and hindgut. Cell bodies (filled circles; not accurate numbers) of neurons are shown in one hemisphere only. The systems displayed are (1) protocerebral neurosecretory cells with axons to corpora cardiaca, anterior aorta and crop duct (CD); (2) subesophageal system (serotonergic) with axons to thoracic-abdominal dorsal neural sheath and several other targets not shown here; (3) thoracic system with terminals in dorsal neural sheath; (4) Lateral abdominal system with axons to pericardial septum of abdominal aorta; (5) median abdominal system with axons to hindgut and sometimes rectal pouch (RP) and its papillae. MT=Malpighian tubules. SEG=subesophageal ganglion.

Table 1. Neuropeptides indicated in the blowfly thoracic-abdominal ganglia[1]

Antisera to	native neuropeptide[2]	distribution
FMRFamide	*CalliFMRFamide 1–13*	IN, NC, EF
pigment-dispersing hormone	PDH-like[3]	IN, EF
proctolin	proctolin[4]	IN, NC, EF, MN
leucokinin I	leucokinin-like	IN, NC
locustatachykinin I	*locustatachykinin-like*[5]	IN
crustacean cardioactive peptide	CCAP-like	EF, IN
corazonin	corazonin-like	IN
allatostatin	*Callatostatins 1–5*	EF[6]
adipokinetic hormone	AKH-like or AKH$^{4-10}$-like[7]	IN, EF, NC?
myomodulin (*Aplysia*)	locustamyotropin-like[7]	NC
galanin (mammalian)	?	IN, NC
galanin message associated peptide	?	IN, NC, EF
enkephalins (mammalian)	?	IN, NC
substance P (mammalian)	? (not locustatachykinin-like)	NC
gastrin/CCK (mammalian)	FaRPs or drosulfakinin-like	IN, NC

Abbreviations: IN=interneurons, NC=neurosecretory cells, EF=efferent neurons, MN=motorneurons

1. Literature references in text. Further neuropeptides have been indicated in ganglia of *Drosophila* (see text)
2. The native peptides in italics have been isolated from *Calliphora*. Others are suggested by analogy with peptides isolated from other insect species.
3. Strong indication for peptide homologous to PDH in *Calliphora* (partial sequence obtained).
4. Proctolin isolated from different arthropods so far is identical (see Ref. [66])
5. Peptides with strong sequence homologies to locustatachykinins have been isolated from *Calliphora* (Lundquist, Holman, Nichols, Nachman, Clottens, Nässel, in press)
6. In *Drosophila* allatostatin immunoreactivity was detected in neurons and neurosecretory cells throughout the CNS.
7. See Schools et al. (Ref. [94])

schematic diagram of Figure 2 highlights the morphology of two types of typical segmental peptidergic neurosecretory cells with terminals in peripheral neurohemal organs. These are the CCAP-immunoreactive (CCAP-LI) type 1 and type 4 neurons of the locust (*L. migratoria*) abdominal ganglia described by Dircksen *et al.* [19]. Terminals of these neurons occur in the distal perisympathetic organs, the lateral heart nerves and the alary muscles associated with the dorsal diaphragm (Figs. 2, 3). Electron microscopy of the CCAP-LI terminals show that they contain large granular vesicles typical of neurosecretory neurons [19] as shown in Figure 3B. The Type 1 and 4 neurons reach the periphery *via* the lateral segmental nerves (N1, N2) of the abdominal ganglia, which is also the case for segmental leucokinin-like immunoreactive neurons [20]. Other putative neurosecretory cells of the locust have axons running dorsally *via* the perisympathetic organs in the median and transverse nerves to the peiphery: locustamyotropin-, FMRFamide- and pancreatic polypeptide-like immunoreactive neurons [27, 60, 94].

In the adult blowfly there are two major neurohemal release sites in the body segments (Fig. 4). One is located in the neural sheath of the dorsal part of the fused thoracic-abdominal ganglion and may correspond to the neurohemal organs of the median and transverse nerves mentioned above. The other is in the pericardial septum or dorsal diaphragm surrounding the abdominal aorta, possibly corresponding to the release sites in the lateral heart nerve and alary muscles of the locust.

NEUROPEPTIDES IN THE NEUROHEMAL AREA IN THE DORSAL NEURAL SHEATH OF THE BLOWFLY THORACIC-ABDOMINAL GANGLION

Although neurohemal areas were known in the neural sheath of thoracic-abdominal ganglia of higher diptera [2, 31], the full extent of the release area in the blowfly neural sheath was first recognized when serotonin immunoreactive (5-HTIR) terminals were revealed in *Calliphora* by immunocytochemistry [68]. The 5-HTIR fibers supply the entire dorsal surface of the thoracic-abdominal ganglion (see Fig. 6D) and also the neural sheath of many of the nerve roots of the ganglion. It was found that these arborizing 5-HTIR fibers are derived from four large neurons in the subesophageal ganglion [68], earlier shown to supply fibers to the neural sheath of ventral nerve roots of the subesophageal ganglion [67]. Later it became apparent that the dorsal neural sheath of the blowfly and fruitfly thoracic-abdominal ganglion also is the termination area of different systems of peptide containing neurons (Figs, 5, 6A, C, Table 2). The first system to be outlined in some detail is formed by six large gastrin/cholecystokinin-like immunoreactive (CCK-LI) ventral neurosecretory cells forming an extensive plexus of fibers in the dorsal sheath of *Calliphora* [65, 72]. This CCK-LI system formed by the ventral thoracic neurosecretory cells

Fig. 5. Myomodulin-like immunoreactive neurons (neurosecretory cells) in the fused thoracic-abdominal ganglion of the adult blowfly. **A.** Ventral view of immunoreactive cell bodies. The six large VTNCs are located in the thoracic neuromeres (T1–3). Four pairs of VANCs are found in the abdominal neuromeres (A1–4). Cc=cervical connective. **B.** Sagittal view of the ganglion displaying the same neurons with their processes. Note terminals in the neurohemal release area dorsally in the neural sheath (NH and arrows). Immunoreactive processes are also found in central neuropil and in axons projecting to the subesophageal ganglion *via* the cervical connective. From Nässel *et al.* [74] with permission.

Table 2. Neuropeptides in neurons and neurohemal areas of blowflies

Putative release site	Location of cell bodies Thoracic neuromeres (T1–3)	Abdominal neuromeres
hindgut		proctolin, FaRPs, PDH, CCAP, CavAS
pericardial septum area	GMAP[1]	proctolin, FaRPs, LK, LVP
thoracic neurohemal area	FaRPs, SP, MM, GAL	
abdominal neurohemal area		MM, FaRPs[2]
fibers in thoracic-abdominal nerves	AKH[1], GAL/GMAP[1]	proctolin, FaRPs, LK, LVP

Abbreviations: FaRPs=FMRFaminde related peptides, PDH=pigment dispersing-hormone-like peptide, CCAP=crustacean cardioactive peptide, CavAS=callatostatins, LK=leucokinin-like peptide, GMAP=galanin message associated peptide-like peptide, LVP=lysinc vasopressin-like peptide (?), SP=substance P-like peptide, MM=myomodulin-like peptide, GAL=galanin-like peptide, AKH=adipokinetic hormone-like peptide

1. The location of the cell bodies is not clear.
2. The "specific" FMRFamide antisera raised in Guinea pig do not label these cells

(VTNCs) was studied in more detail and turned out to be immunopositive with a number of antisera to non-insect peptides: bovine pancreatic polypeptide, CCK, methionine enkephalin, methionine enkephalin-Arg-Phe [21], FMRFamide, molluscan small cardioactive peptide (SCP_B) (Fig. 6), substance P [50] and the molluscan peptide myomodulin [74] (Fig. 5). It is likely that the VTNCs contain peptides of the CalliFMRFamide series which have been isolated from *Calliphora* thoracic-abdominal ganglia [22] and it is possible that many of the antisera listed above cross react with different epitopes of the CalliFMRFamides. In *Drosophila* the homologs of the VTNCs can also be identified by antisera against FMRFamide [50, 106, 109]. Several FMRFamide-related peptides (FaPRs) have been isolated from *Drosophila* tissue or deduced from isolated cDNAs [64, 75-77, 92] and the FaRPs appear to be the products of three different genes [76]. It was shown by *in situ* hybridization histochemistry that the message encoding one of the FMRFamide precursors is expressed in the *Drosophila* VTNCs [78, 93]. In addition to FaRPs it is possible that the VTNCs contain colocalized

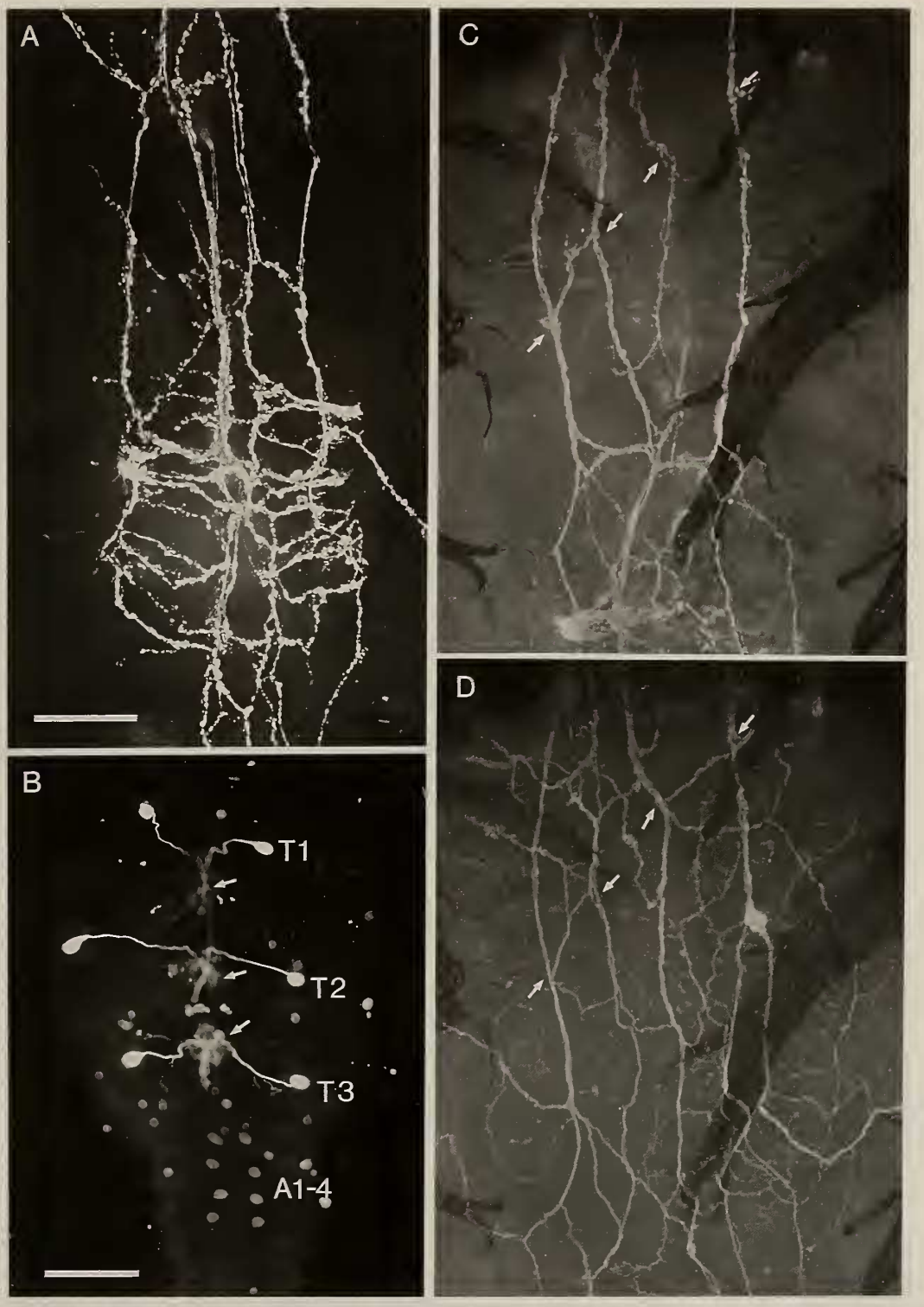

FIG. 6. Fluorescence micrographs of thoracic-abdominal neurons in wholemounts of the blowfly. **A.** The neurohemal plexus in the dorsal neural sheath of the thoracic-abdominal ganglion labeled with monoclonal antibody to SCP_B. **B.** The six cell bodies (T1–3) and segmental neurohemal release sites (arrows) of the VTNCs of a pupal blowfly (48 h pupa). At this stage the segmental organization of the release sites is still apparent. Antiserum to SCP_B. **C** and **D.** Double labeling of the same wholemount with antisera against FMRFamide (**C**) and serotonin (**D**) viewed with filters for fluorescein (FITC) and Texas red (using biotin-streptavidin detection). The fibers in the FMRFamide immunoreactive plexus are closely adjacent to the serotonergic plexus (see arrows for correlation) along the ganglion midline. Scales A, C, D = 50 μm; B = 100 μm.

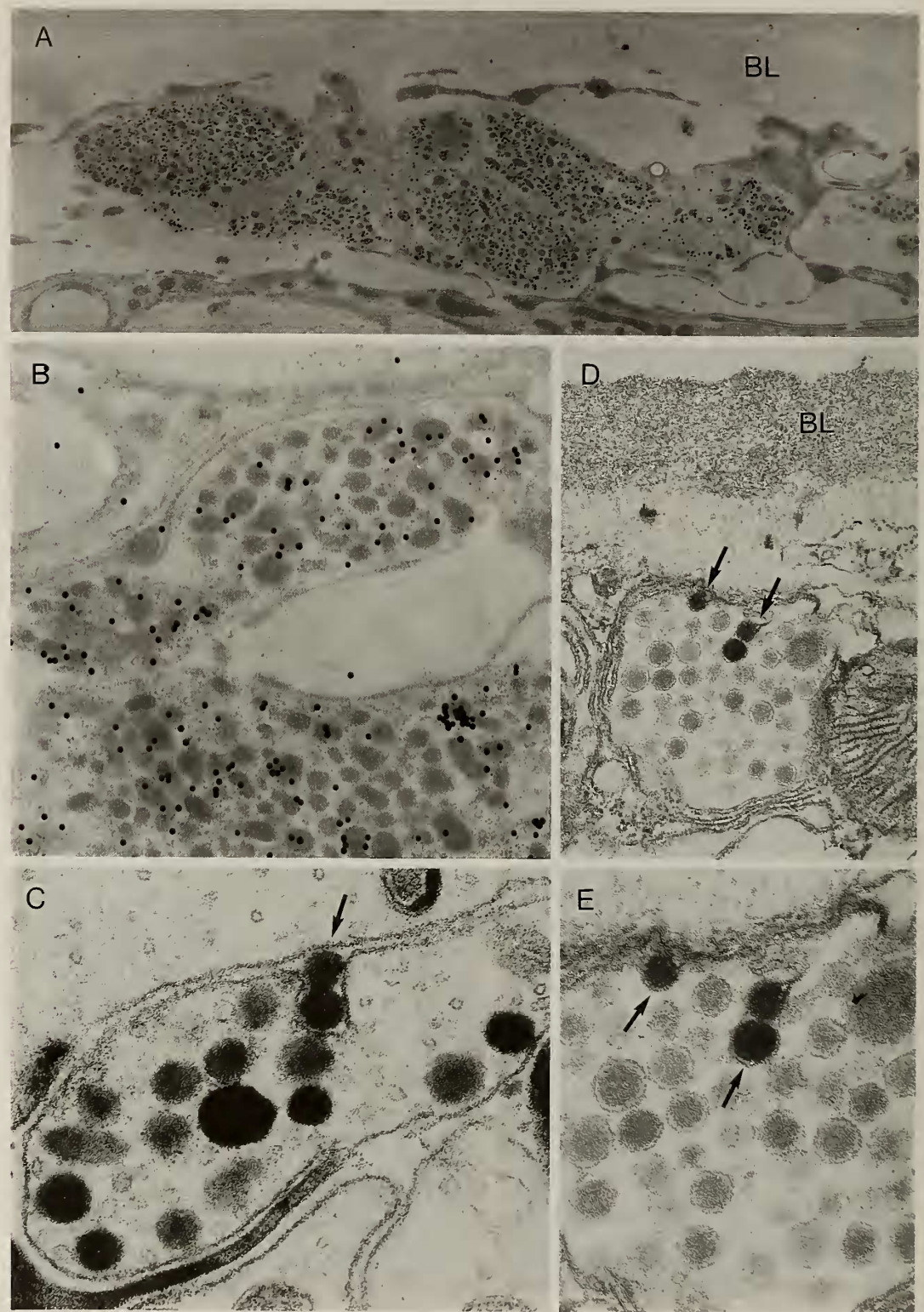

Fig. 7. Electron microscopy of peptidergic terminals in the dorsal neural sheath of the thoracic-abdominal ganglion of *Calliphora*. **A.** Immuno-gold labeling to display cluster of FMRFamide immunoreactive terminals in the sheath just below the acellular basal lamina (BL). **B.** Detail in higher magnification of FMRFamide immunoreactive terminals with large granular vesicles. **C.** Conventional electron microscopy of exocytotic profile (arrow) in one of the peptidergic terminals in the sheath. Two peptidergic vesicles are being released. **D and E.** With the TARI method exocytosis is more easily detected. Here three vesicles (arrows) are being released by a peptidergic terminal. With the TARI method the exocytosing vesicles become osmophilic and hence stand out (arrows in D). BL=the acellular basal lamina. E is a higher magnification of terminal in D. Magnifications: A=14.000×, B=46.000×, C=86.000×, D=36.000×, E=72.000×.

peptides related to substance P and myomodulin (or locustamyotropins). A recent study of larval and adult *Drosophila* has indicated the presence in the VTNC homologs of peptides reacting with antisera to *Manduca* allatotropin and allatostatin [111]. The same authors showed similar cells with terminals in the dorsal neurohemal area reacting with antisera to *Bombyx* PTTH and *Manduca* diuretic hormone.

The peptidergic neurohemal plexus formed by the VTNCs is not as extensive as the one formed by the 5-HTIR cells. The plexus of the VTNCs is restricted to a median portion of the sheath of the dorsal thoracic-abdominal ganglion, whereas the 5-HTIR plexus covers the entire dorsal surface (Fig. 6C, D). Double labeling experiments with 5-HT- and FMRFamide- or SCP_B antisera revealed that the fibers of the VTNCs and the 5-HTIR fibers are located adjacent to each other in the plexus of the median region of the dorsal neural sheath (Fig. 6C, D).

The VTNCs also arborize extensively within the thoracic neuropils (Fig. 5) and each cell sends an axonal process anteriorly to the subesophageal ganglion [50]. It has not been determined whether any of the arbors represent input regions of the neurons. The possibility exists that the dendritic arbors of the VTNCs were not immunolabeled, by analogy with the vasopressin-like immunoreactive neurons of the locust subesophageal ganglion where the exclusion of such immunostaining was determined by intracellular dye injection [100].

Additional neurosecretory cells forming terminals in the dorsal neural sheath are found in the abdominal ganglion. Most clearly this was seen for ventral abdominal neurosecretory cells (VANCs) labeled with an antiserum to the molluscan neuropeptide myomodulin [74] (Fig. 5). Myomodulin [15] shares the C-terminus -RLamide with the locustamyotropins I-IV [94] and probably the myomodulin antiserum recognizes native blowfly peptide(s) of myotropin type. Therefore it is not surprising that in the locust locustamyotropin antiserum labels abdominal neurosecretory cells with terminals in median nerve perisympathetic organs [94]. In *Calliphora* and *Phormia* double labeling experiments with antisera to myomodulin and FMRFamide (raised in Guinea pig) and SCP_B reveal that the myomodulin immunoreactive VANCs in the abdominal ganglion, do not contain epitopes recognized by the SCP_B and the more specific FMRFamide antiserum. A dense myomodulin-like immunoreactive plexus extends over the entire abdominal portion of the neural sheath, whereas in the same specimens the plexus labeled with SCP_B or FMRFamide antisera is more insignificant in the abdominal portion. Thus it is possible to determine that most of the peptide containing fibers in the most caudal portion of the neurohemal plexus in blowflies are derived from the abdominal neurosecretory cells. A separate plexus of fibers in the thoracic-abdominal sheath derived from cell bodies distinct from the VTNCs was labeled with an antiserum against the mammalian neuropeptide galanin [51].

The only substance that so far has been shown to be released from the thoracic-abdominal ganglion of *Calliphora* by high potassium depolarization is serotonin; the likely role of the released serotonin is to induce secretion in the salivary glands [101]. No experimental evidence for peptide release from thoracic-abdominal neurohemal areas are available for blowflies, but the peptide immunoreactive terminals in the neural sheath are located outside the blood brain barrier [21, 72] (Fig. 7), like the serotonergic terminals [68]. For indirect demonstration of peptide release we applied the tannic acid ringer incubation (TARI) method [7] on living ganglia *in vitro* to augment the detection of exocytosis (diagnostic of release) in peptidergic terminals in the sheath of the thoracic-abdominal ganglion. The dissected ganglia were left in a dish with 0.5% tannic acid in insect saline for 2 hr followed by fixation in glutaraldehyde and osmium [3]. The TARI method renders the core of extruded peptidergic vesicles osmophilic (Figs. 7D, E) which facilitated the detection of numerous exocytosis profiles in peptidergic fibers of the *Calliphora* thoracic-abdominal neurohemal release site (cf. Fig. 7C).

What are the actions of neurohormones released from the neurohemal area in the thoracic-abdominal ganglion? It is presumed that the serotonin released from this area induces fluid secretion in the blowfly salivary gland [10]. It is also known that serotonin can induce diuresis and modulate activity of heart, visceral and oviduct muscles [13, 14, 54, 58]. For the FaRPs some clues have been obtained from *in vitro* studies on *Calliphora* salivary glands: three of the CalliFMRFamides induce fluid secretion in the salivary glands [22]. If this action is physiological it is likely that the peptide(s) reach the salivary glands *via* the circulation like serotonin does. Duve *et al.* [23] have also demonstrated that two of the CalliFMRFamides increase the spontaneous activity of the abdominal heart. Since a direct innervation of the abdominal heart has been demonstrated (see below), it is not clear whether hormonally released peptide is involved in this action. The FaRPs, if released into the circulation, can reach a whole host of peripheral targets all of which need to be tested for their response.

PEPTIDES IN THE NEUROHEMAL AREA IN THE PERICARDIAL SEPTUM OF THE BLOWFLY

The organization of the neurohemal area of the pericardial septum (dorsal diaphragm) was first described in the stable fly by electron microscopy [57]. The first identified neurons innervating this neurohemal area were detected in the blowfly with antiserum against the cockroach myotropic peptide leucokinin I [10]. The leucokinin-like immunoreactive (LK-LI) fibers reach the septum *via* the segmental abdominal nerves and are derived from a set of about 20 cell bodies in the abdominal ganglion (Figs. 4, 8A, 9A, B). These cell bodies also form central processes within the median portion of the abdominal neuropil. It is likely that these central processes represent release sites within the neuropil, but it cannot be excluded that they also receive synaptic inputs in this region. Interestingly the cell bodies

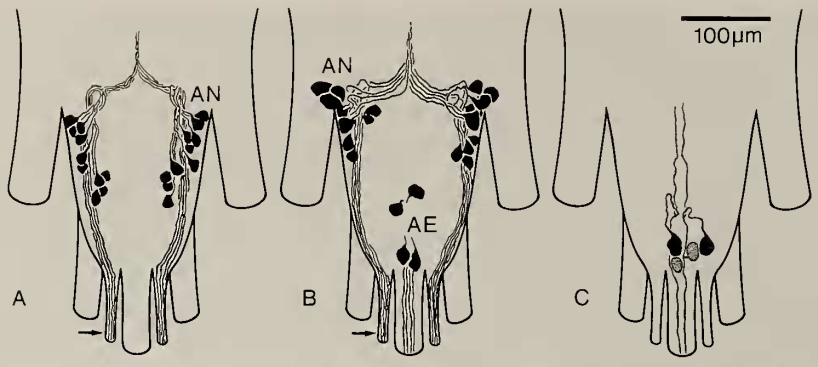

Fig. 8. The abdominal portion of the blowfly thoracic-abdominal ganglion with efferent peptidergic neurons. **A.** Antiserum to leucokinin I labels about 20 neurons (AN) with axons to pericardial septum (altered from [10]). **B.** Antiserum to proctolin labels a similar (but not identical) set of 20 efferents with axons to the pericardial septum and a set of four caudal abdominal efferent neurons (AE) with axons to the hindgut (altered from [69]). **C.** An antiserum to CCAP labels four cells posteriorly in the abdominal neuromeres. At least two of these send axons to the hindgut (Nässel and Dircksen, unpublished).

and fibers of the 20 LK-IR efferents could also be immunolabeled with an antiserum to lysine vasopressin [71]. Similar abdominal LK-LI efferents in the cockroach *Leucophaea maderae* were also found lys-vasopressin immunoreactive [70] (see also Ref. [17]). The native blowfly peptide(s) related to the cockroach leucokinins have not yet been isolated, but radioimmunoassays of blowfly tissue extract indicate the presence of leucokinin-immunoreactive material that eluted in HPLC in two zones, one of which has the same retention time as synthetic leucokinin I [53]. The biological actions of leucokinin-related peptides in blowflies are not known. Although present in fibers in the lateral cardiac nerves of *L. maderae* [70], none of the leucokinins are cardioactive in this cockroach [39]. Possibly these peptides are instead released in into the circulation *via* the pericardial septum in *L. maderae* and blowflies, and have targets elsewhere. Clues to such targets have been obtained from studies of the actions of some of the leucokinins and achetakinins in some other insects by *in vitro* assays. It is clear that apart from myotropic actions of the leucokinins at the cockroach foregut, hindgut and oviduct [39], some of the achetakinins and leucokinins can regulate fluid secretion in the Malpighian tubules of the mosquito *Aedes aegypti* and the cricket *Acheta domesticus* [30, 35]. It is to be expected that the leucokinin-related peptides from different insect species have diverse physiological roles (as neurohormones, as well as modulators within the CNS). A calcium dependent release of leucokinins could be induced *in vitro* from the corpora cardiaca of *L. maderae* by potassium depolarization [59], but was not yet tested for abdominal neurohemal areas. The hemolymph of *L. maderae* was also shown to contain leucokinin immunoreactive material in the nanomolar range [59], indicating that leucokinins may be released as hormones *in vivo*.

FMRFamide-like immunoreactive (F-LI) fibers have been detected in the wall of the abdominal aorta and the pericardial septum of *Calliphora*. The origin of these fibers was not determined, but they are probably originating from cell bodies in the abdominal neuromeres. As noted above, two of the CalliMRFamides increase the spontaneous heart rhythm [23] and it is possible that this effect is by peptide released from the F-LI efferents innervating the heart.

Proctolin-like immunoreactive (P-LI) material has been detected in a relatively large number of neurons in the blowfly thoracic-abdominal ganglion [69]. Two sets of efferent P-LI neurons have cell bodies located in the abdominal neuromeres (Fig. 8B). They send axons *via* the median and lateral abdominal nerves respectively. The approximately 10 P-LI neurons located laterally in each side of the abdominal ganglion send axons *via* the lateral abdominal nerves to the pericardial septum. Proctolin is known to increase the heart beat in a number of insect species [79], but no records of activity in flies exist. In different dipteran insects myogenic actions of proctolin have, however, been noted in hindgut and oviduct, and in larval body wall muscle [79].

Recent experiments reveal that an antiserum to the mammalian peptide galanin message associated peptide (GMAP) labels an extensive plexus of varicose fibers in the pericardial septum of *Calliphora*. So far, no biological action for this peptide has been recorded for any organism (Lundquist *et al.*, 1992), but the action of GMAP on the blowfly heart can be probed quite easily. The cellular origin of the GMAP immunoreactive fibers in the septum has not been determined.

Antisera to three more peptides, crustacean cardioactive peptide (CCAP), corazonin and pigment-dispersing hormone (PDH), were tested on the dorsal abdominal diaphragm and pericardial septum. Although the first two of these are cardioactive in other insects [11, 19, 41, 48, 107], no immunolabeling was obtained with any of the three antisera in the blowfly pericardial septum. Serotonin immunoreactive fibers could, however, be detected in the pericardial septum of *Phormia*. These fibers are likely to be derived from the four large subesophageal cell bodies, *via* the superficial fiber system in the neural sheath emerging also through the sheath of the lateral abdominal nerves. A direct action of serotonin on the heart or alary muscle as indicated in other insects is

possible [58].

In conclusion it is not clear whether the substances indicated by immunocytochemistry in fibers in the blowfly pericardial septum act on the heart muscle (and alary muscles) or if they are released into the circulation for action elsewhere (or both). An exception may be the F-LI fibers since some of the CalliFMRFamides have been shown to be cardiactive in *Calliphora* [23].

VARICOSE AXONS IN THE NEURAL SHEATH OF PERIPHERAL NERVES

In several insect there are plexuses of varicose fibers in the neural sheath of several of the peripheral nerve roots. For instance antisera against FMRFamide, pancreatic polypeptide, glucagon, adipokinetic hormone (AKH) and CCA label plexuses of varicose fibers in the perineurium of segmental and link nerves of crickets and locusts [19, 61, 86, 96, 99] (see also Fig. 2). Some of these fibers are derived from cell bodies in the CNS, others from peripherally located neurosecretory cells. In blowfies there are also systems of varicose fibers in the neural sheath of some of the peripheral nerves. These fibers have in some cases been traced from abdominal cell bodies to the periphery where they form terminals in neurohemal release areas: fibers in sheath of the lateral abdominal nerves, destined for the pericardial septum, react with antisera against antisera against leucokinin [10], proctolin, FMRFamide and lysine vasopressin [9, 69, 61]. In the sheath of the anterior prothoracic-, anterior dorsal mesothoracic- and haltere nerves superficial fibers react with antisera to AKH and the mammalian neuropeptides galanin and galanin message associated peptide (GMAP) [51, 52]. The origin of these fibers has not been determined. Furthermore, 5-HTIR fibers from cell bodies in the subesophageal ganglion were seen in the sheath of most cephalic and thoracic-abdominal nerve roots [67, 68].

NEUROPEPTIDES IN EFFERENT NEURONS TO THE BLOWFLY HINDGUT

The blowfly hindgut is innervated by abdominal neurons *via* the median abdominal nerve (Fig. 4). As will be shown below, several neuropeptides have been indicated in some of these efferent abdominal neurons by immunocytochemistry with antisera to proctolin, FaRPs, PDH, callatostatin and CCAP. It is not clear whether these peptides act on hindgut motility, water and ion balance or are released as neurohormones into the circulation around the intestine (or have several functions).

Proctolin. Proctolin was isolated on basis of its myotropic action on the cockroach hindgut [98], and early on proctolin was detected immunocytochemically in six efferent abdominal neurons with terminals in muscle of the hindgut of *Periplaneta americana* [25]. It has also been shown that proctolin increases the frequency and amplitude of myogenic contractions of the hindgut of a dipteran insect, the stable fly *Stomoxys calcitrans* [37]. In blowflies four abdominal proctolin immunoreactive (P-LI) neurons (Fig. 8B) send axons *via* the median narve to the hindgut where P-LI terminals could be found on the hindgut, rectal valve, rectum and rectal papillae [9]. Some of the P-LI fibers innervate the muscular is of the intestine and in the rectal papillae the fibers invade the medullary region. The P-LI fibers may hence mediate control of muscle activity as well as regulation of water and ion balance.

FMRFamide related peptides. Efferent F-LI nuerons of the abdominal ganglion send axons *via* the median abdominal nerve to the hindgut where they form an innervation pattern very similar to that of the proctolin containing neurons, but with larger number of arborizations [9, 50]. The Fa-LI fibers innervate the hindgut (Fig. 9C), rectal valve, rectum and rectal papillae. Also FaRPs may have myotropic actions on the hindgut and/or be involved in the regulation of water and ion balance.

Pigment dispersing hormone (PDH). Members of the pigment dispersing hormone family of peptides have been isolated from a number of crustacean and insect species [87] and a partial amino acid sequence (12 of 18 amino acids) of a *Calliphora* peptide with strong homologies to β-PDH has been obtained (Lundquist *et al.*, in prep.; see also Ref. [73]). In blowflies there are neurons reacting with PDH antiserum in the brain and thoracic abdominal ganglion [73]. In the abdominal ganglion six PDH-LI neurons send axons to the hindgut where they form varicose terminals in the posterior region of the midgut and anterior portion of the hindgut (Fig. 9D), the rectum and the rectal papillae [73]. The six PDH-LI neurons also have varicose arborizations within abdominal neuropil. An additional release site for PDH-related peptide may be the wall of the anterior aorta where PDH-LI terminals, derived from cephalic neurosecretory neurosn, are found [73].

Crustacean cardioactive peptide (CCAP). The nonapeptide CCAP was first isolated from the crab *Carcinus maenas* and later in identical form from the insects *Locusta migratoria* and *Manduca sexta* [11, 48, 97]. In the locust CCAP immunoreactivity (CCAP-LI) was demonstrated in numerous neurons and neurosecretory cells as seen in Figures 1 and 2 [19]. Some of these supply fibers to segmental distal perisympathetic organs and neurohemal release sites in the dorsal diaphragm including the alary muscles and lateral heart nerve [19]. In adult blowflies there are only four CCAP-LI cells in the entire fused thoracic-abdominal ganglion, two of which are only weakly immunoreactive (Fig. 8C). These cells are located posteriorly in the abdominal portion of the ganglion and send axons to the hindgut via the median abdominal nerve (Fig. 8C). The CCAP-LI fibers supply only the hindgut and rectum, but not the rectal papillae or pouch. Similar CCAP-LI neurons occur in adult *Drosophila* (Dircksen and Breidbach, unpublished).

Allatostatin-related peptides (callatostatins). Five neuropeptides termed callatostatins 1–5 have been isolated from adults of the blowfly *Calliphora vomitoria* [24]. Two of

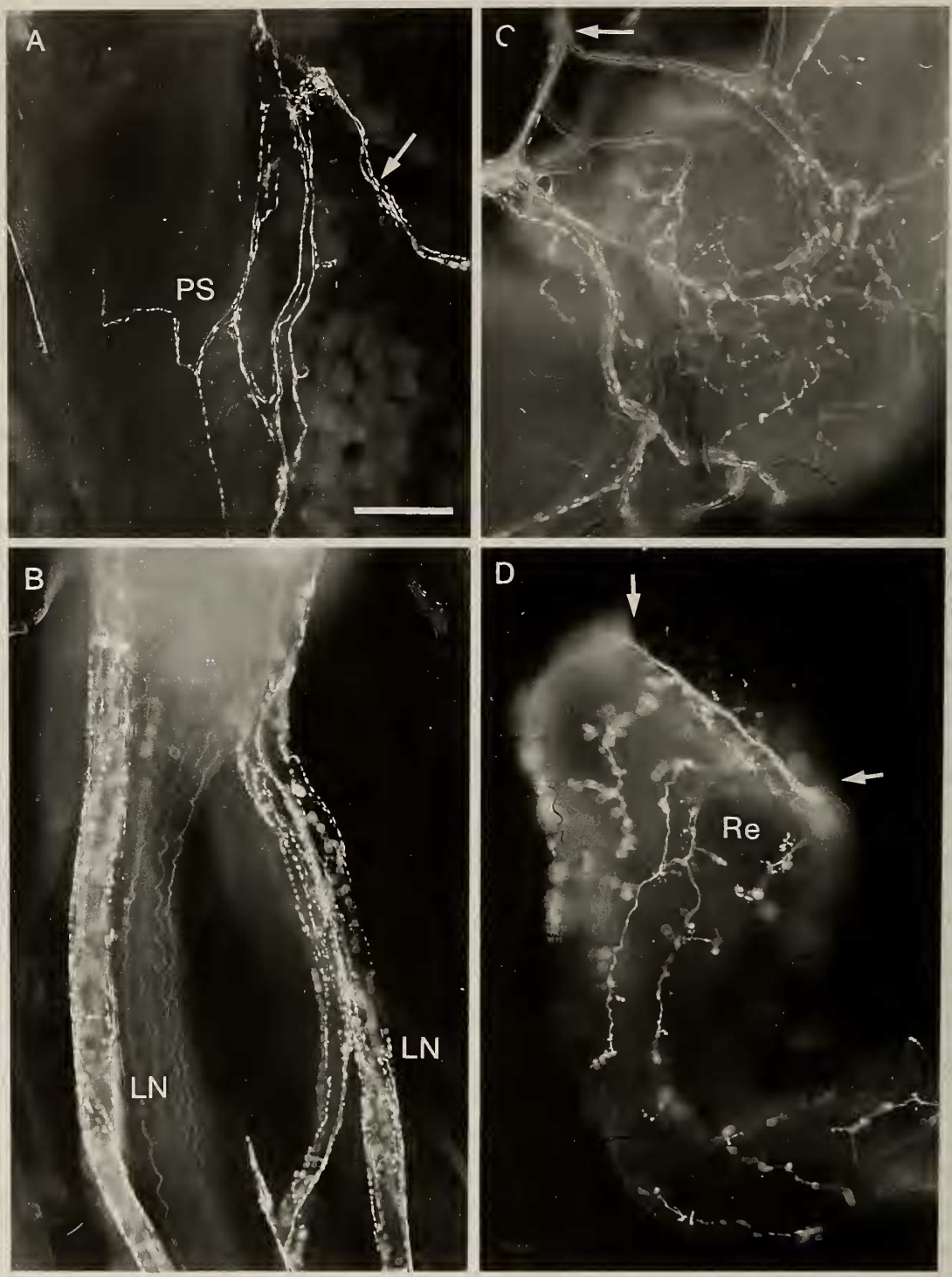

Fig. 9. Fluorescence micrographs of peripheral peptidergic axons in blowfly. **A.** Leucokinin immunoreactive terminals in the pericardial septum (PS). The fibers arrive by the segmental nerves (one indicated by arrow). **B.** Leucokinin immunoreactive varicose fibers at the surface of the lateral abdominal nerves (LN) on route to the pericardial septum. **C.** FMRFamide immunoreactive fibers in the hindgut anterior to rectal valve. The axons arrive from the abdominal nerve at arrow. **D.** PDH immunoreactive fibers in first part of the rectum (Re) of the hindgut (rectal valve at arrows). Scales: A-D = 50 μm. A-D altered from [9, 10, 73].

these were specifically isolated from dissected thoracic-abdominal ganglia. In the cockroach *Diploptera punctata* the cockroach allatostatins as well as the fly callatostatins inhibit juvenile hormone production in the corpora allata *in vitro*, whereas in the adult blowfly these peptides have no action on the production of juvenile hormone in the corpora allata [24]. In accordance with this Duve *et al.* [24] found no callatostatin immunoreactive material in the corpora allata or

any of the neurosecretory cell systems of the blowfly nervous system. Instead, callatostatin immunoreactivity was found in neurons of the abdominal ganglion with axons emerging through the median abdominal nerve to terminals in the hindgut, rectum, rectal papillae and oviduct. These authors therefore suggest that functions other than allatostatic ones may have to be sought for the callatostatins in adult blowflies. In sharp contrast to the findings of Duve *et al.* [24], allatostatin-like immunoreactivity was, however, found in neurons and neurosecretory cells both in the brain and thoracic-abdominal ganglion of *Drosophila* by Zitnan *et al.* [111]. It is not clear whether this discrepancy is caused by species differences or methodological differences.

LARVAL NEUROHEMAL ORGANS AND PERIPHERAL RELEASE SITES IN BLOWFLIES

In the larvae of higher diptera the corpora cardiaca, corpora allata and prothoracic gland form a composite organ, the ring gland or Weissman's ring [108]. In *Calliphora* serotonin and gastrin/CCK immunoreactive cell bodies and fibers were detected in the ring gland [8]. Additionally cephalic neurosecretory cells reacting with antisera against gastrin/CCK [8], FMRFamide, PDH (Nässel, unpublished) and corazonin (Cantera, Veenstra, Nässel, in prep.) form plexuses of varicose axons in the wall of the anterior portion of the aorta.

The presence of neurohemal release sites in the larval thoracic-abdominal nervous system of *Drosophlia* and *Calliphora* was first indicated by immunocytochemistry with antisera against FMRFamide and gastrin/CCK [65, 72, 101, 109]. In these flies each of the three thoracic segments have a dorsal unpaired median nerve which contributes to an extended bulb-like neurohemal organ [65, 72] (Fig. 10). In some specimens there is one neurohemal organ on each dorsal unpaired nerve, in others these fuse to one or two organs. FMRFamide-, myomodulin- and CCK-like immunoreactive fibers invade these thoracic neurohemal structures *via* the dorsal unpaired nerves (Fig. 11A) and peptidergic terminals could be revealed in the neural sheath outside the blood brain barrier [72]. The immunoreactive fibers are derived from two large cell bodies ventrally in each thoracic segment (Fig. 11A). By analysis of the postembryonic development of these immunoreactive cells it could be demonstrated that they persist throughout metamorphosis (see Fig. 6B) and form the six VTNCs of the adult blowfly and fruitfly [72, 101]. Interestingly, it can thus be concluded that the segmentally organized larval thoracic neurohemal organs transform into a large fused neurohemal area. There are also varicose myomodulin immunoreactive fibers emerging through the first four dorsal unpaired nerves (A1-4) of the abdominal ganglion in larvae of *Calliphora* (Fig. 11A) and *Phormia* (Nässel, unpublished). These nerves bifurcate laterally and the immunoreactive fibers continue along the length of the branches. The fibers entering the first four abdominal dorsal median nerves are derived from five pairs of ventral myomodulin immunoreactive cell bodies in abdominal neuromeres A1-5 (Fig. 11A). Four of these probably correspond to the myomodulin immunoreactive VANCs seen in the adults. As mentioned earlier the larval VTNCs of *Drosophila* were shown to contain peptide reacting with antisera to allatostatin and allatotropin [111].

Peptide containing cells with abdominal cell bodies and peripheral axonal projections can also be detected in the larval blowflies. Some of them have been followed through metamorphosis. The neurons of interest react with antisera

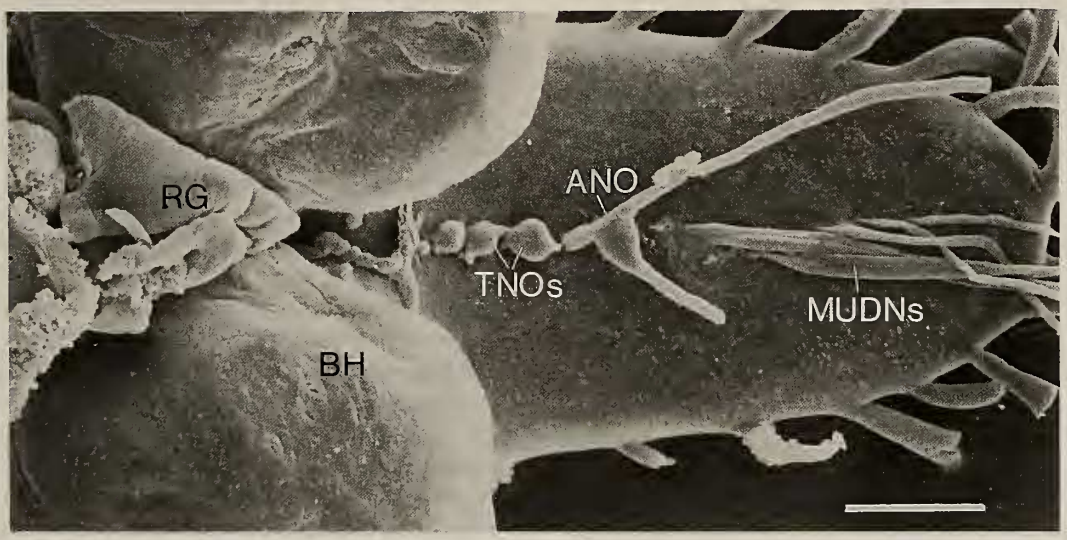

FIG. 10. The larval nervous system of *Calliphora*. Scanning electron micrograph of the larval nervous system in dorsal view. The three thoracic neurohemal organs (TNOs) are seen as spherical structures. A neurohemal organ (ANO) is also formed by the first abdominal dorsal unpaired nerve. The remaining abdominal median unpaired dorsal nerves (MUDNs) also contain some varicose fibers from neurosecretory cells (myomodulin immunoreactive). Altered from [72]. BH=brain hemishere. RG=portion of the ring gland. Scale=100 μm.

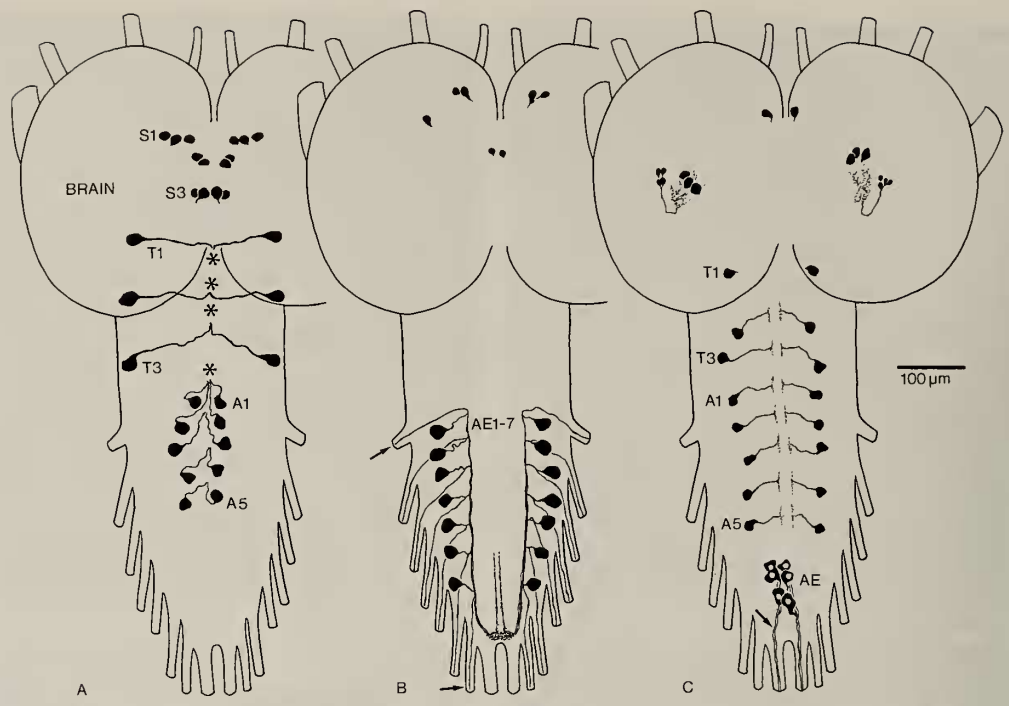

Fig. 11. Peptidergic neurons in the larval blowfly nervous system. **A.** Myomodulin immunoreactive neurons located ventrally (some dorsal cells in the brain are not shown). Neurons are found in two of the three subesophageal segments (S1 and S3). The three VTNCs are seen in segments T1–T3. These cells send their axons to the three median neurohemal organs of the thoracic neuromeres (location indicated by asterisks). Five pairs of ventral abdominal neurosecretory cells (A1–5) send their axons into the dorsal unpaired median nerves (location of one indicated by asterisk). **B.** Leucokinin immunoreactive neurons in the brain and abdominal neuromeres. The seven pairs of abdominal neurons (AE1–7) send axons through the segmental nerves 1–7 (arrows). The axons terminate on segmental abdominal body wall muscles. **C.** Pigment dispersing-hormone immunoreactive neurons in brain and thoracic-abdominal ganglia. The 8 pairs of thoracic and abdominal neurons (T1–3 and A1–5) appear to be interneurons, whereas three pairs of posterior abdominal neurons (AE) are efferents probably destined for the hindgut. A-C (Nässel, unpublished).

against proctolin, lysinevasopressin [71], leucokinin [10] and PDH (Nässel, unpublished).

The leucokinin (and lysine vasopressin) immunoreactive cells of the larva are segmentally distributed laterally in the abdominal neuromeres A1-7 (one pair of cells per neuromere, except in A8; Fig. 11B). These cells form efferent axons projecting to segmental muscle of the abdominal body wall of the larva [10]. The abdominal LK-LI and vasopressin immunoreactive cells survive metamorphosis and their peripheral axons innervate the pericardial septum as described above [10, 71].

A proctolin antiserum labels four cells with axons in the median abdominal nerves (A8) and seven pairs of lateral neurons with axons emerging through the lateral nerves (A1-7) in the blowfly and fruitfly larvae [1, 71]. In *Drosophila* the lateral cells send axons to body wall musculature and in both insects the median one innervate the intestine [1, 71]. Both sets of abdominal P-LI cells survive metamorphosis and in adults supply the pericardial septum and hindgut respectively.

In the blowfly larva six efferent PDH-LI neurons can be seen in the caudal portion of the abdominal ganglion (Fig. 11C). It has not yet been determined whether these cells survive metamorphosis and transform into the six cells of the adults that innervate the hindgut [73].

In summary, it can be proposed that many of the neurosecretory and efferent cell systems of the blowfly are present already in the larva. The cells survive metamorphosis, attain slightly altered morphologies and form novel release sites. It is not known whether the functions of the larval neurosecretory systems and the peptides they release are retained into the adult organism or if they obtain new actions.

CONCLUSIONS

Insects possess an impressive set of multiple specialized neurohemal release sites in different portions of the head and body compartments. Raabe [86] suggested that the multiple release sites are necessary due to the poorly developed circulatory system of insects. Thus it is not unusual to detect putative release sites for the same neuropeptide in the corpora cardiaca, the thoracic and abdominal perisympathetic organs and in the abdominal heart region (pericardial septum and alary muscles) [19, 49]. In this account we have shown that also in flies such as *Calliphora, Phormia* and *Drosophilla* there are several neurohemal release sites in addition to the corpora cardiaca. In fact, in these flies the

neurohemal part of the corpora cardiaca is insignificant in size compared to the neurohemal release sites in the neural sheath of the thoracic-abdominal ganglion and the pericardium. Thus the release of peptides from corpora cardiaca and the attending neurohemal plexus in the anterior aorta may be small in comparison with that of neurohemal release sites in structures derived from the body segments.

Many of the neurosecretory cells in addition to peripheral release sites have putative release sites within neuropils of the central nervous system. An interesting example is provided by the eclosion hormone containing neurons of larval moths (*Manduca sexta*). These neurons have varicose axons running through the entire length of the cephalic and thoracic-abdominal ganglia before they reach their neurohemal release sites on the hindgut [103]. Another example are the blowfly and *Drosophila* VTNCs which have extensive central arborizations in the subesophageal and thoracic neromeres as well as terminals in a substantial neurohemal release site [50]. Release of peptides or monoamines by the same neurons at different peripheral neurohemal release sites in addition to central neuropil regions would ensure synchronous action on (regulation of) peripheral targets and central circuits and thus enable orchestration of behavioral routines or other physiological functions [5, 45, 104, 105].

Some peptidergic neurons, that were originally classified as neurosecretory cells have all their known processes within the central nervous system [88, 100]. Are they still to be considered as neurosecretory cells? With the data available today on the distribution of a large number of different neuropeptides in many types of interneurons it may be called for to be cautious about terminology. Scharrer [90] noted that neurons employing chemical messenger substances have secretory capacity and may, in case of interneurons, release their regulatory substances in certain distinct neuropils without forming typical synaptic contacts. However, with the emergence of the concept of colocalized neuropeptides and classical transmitters [49] the classification into neurosecretory cells and neurons may be obsolete. Neuropeptides in central neurons may have a host of actions as neurotransmitters, cotransmitters, neuromodulators or even as trophic factors [36] and in insects we are only just starting to learn about central functions. Neuropeptide release often is episodic [36, 90, 104, 105] and many neuropeptides act in pacemaker circuits [34] indicating that central roles of neuropeptides are in regulation of rhythmic events or triggering of innate behaviors.

A large number of neuropeptides have been isolated from crustaceans and insects [38, 42, 55, 56, 94], and probably most of them have some actions at peripheral targets. Why are so many peptides needed in a "simple" organism like an insect? It is likely that many regulatory processes are finely tuned and that this requires several chemical messengers. Studies on diuresis in insects have for example provided evidence that more than one peptide may be involved only at the level of the Malpighian tubules [4, 30]. In the cricket A. *domesticus* fluid secretion can be induced by two different types of neuropeptides, achetakinin I and the 46 amino acid diuretic peptide [30]. The two peptides act *via* different receptors and second messenger systems in the Malpighian tubules, achetakinin by an unknown pathway and diuretic peptide *via* cAMP. Additionally serotonin acts on cricket Malpighian tubules [12] indicating that diuresis is a finely tuned proces in insects, notwithstanding further medhanisms involved in water reabsorption in the hindgud (See Ref. [4]). We also know that a variety of neuropeptides in addition to monoamines act on skeletal, visceral and heart muscle in insects [16, 82, 94]. For instance the spontaneous contractions of locust oviduct muscle appear regulated by a number of neuropeptides as well as by octopamine [46, 47, 82, 94] and a very large number of neuropeptides and serotonin act on the *Leucophaea* hindgut [39, 94]. The role of insect hormonal neuropeptides in regulation and initiation of behaviors has been explored for eclosion hormone and cardioactive peptides during development of the moth *Manduca* [104, 105]. It is to be expected that for instance feeding behavior is under peptide hormone control since salivary glands, intestinal muscle and skeletal muscle are regulated by different peptides and central circuits associated with feeding are innervated by peptidergic neurons [16, 22, 66, 80, 94]. Peptides originating from cells outside the nervous system have also been implicated in behavior regulation. As an example the *Drosophila* 36 amino acid sex peptide of the male accessory glands is transferred to females during copulation and elicit rejection of further males as well as an ovulation and oviposition response [91].

Characterization of receptors mediating the action of different neuropeptides and studies of the structure of the active cores of neuropeptides have been initiated [30, 44, 56, 62, 85], but much is to be learned about interactions of different peptides at the receptor and second messenger level. We also need to know more about the degradation of peptides at their target organs such as Malpighian tubules, hindgut and ovaries [83, 84] or within the hemolymph [29]. These studies are necessary to be able to determine whether peptides released from neurohemal organs will be available in physiological concentrations for actions at their targets (at threshold concentrations indicated in *in vitro* assays) or if they are likely to act by direct release from neuronal terminals supplying the targets. It is also critical to employ sensitive assays such as radioimmunoassay or ELISA to determine the content of peptides in the hemolymph in *in vivo* experiments to corroborate claimed hormonal actions.

Analysis of neuroendocrine systems at the molecular and genetic level will no doubt be helpful in filling many gaps in the understanding of hormonal control of development, behaviour and homeostasis [6, 95]. It is to be expected that some of the diversity in neuropeptides and peptides in non-neural cells will be explained by a certain redundancy of mediators in regulatory systems which is partly caused by a need for subtle control mechanisms. It is also likely that peptides are involved in processes other than classical neurotransmission, hormonal actions or neuromodulatory roles,

such as being cytokines or factors in development and maintenance of the nervous system.

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